## REMARKS

Applicants acknowledge that claims 76-78, 81, 86-88, 91, 106, 108, 110, 112 and 115-123 were pending and under examination. Claims 76, 78, 81, 86, 88, 91, 106, 110, and 112 are currently amended. Support for the claims amendments can be found at least, for example, at page 2, line 21 through page 3, line 20, in particular, at page 3, lines 9-11 and 17-19, of the specification as originally filed. No new matter has been added.

### *Interview*

Applicants thank Examiners Wollenberger and Schultz for conducting a personal interview with Applicants' representatives on June 26, 2007. Issues related to the rejections under 35 USC 112, 102/103, and obviousness-type double patenting, as set forth in the Office Action, were discussed. Proposed claim amendments were also discussed.

### *Double Patenting*

Claims 76-78, 81, 86-88, 91, 106, 108, 110, 112 and 115-123 are provisionally rejected on the grounds of nonstatutory obviousness-type double patenting as being unpatentable over claims 1, 4, 7, 8, 13, 14, 20, 25, 27 of copending Application No. 10/255,568 and new claims 30-49 of copending Application No. 11/142,866. In addition, the Examiner notes that the following applications *may* claim the same or similar subject matter: 10/832,248; 10/638,253; and 10/832,432.

Applicants respectfully direct the Examiner's attention to the fact that a Response was filed by Applicants in the commonly-owned, co-pending Application No. 10/255,568. Applicants cancelled non-elected claims 1, 4, 7, 8, 13, 14, and 27. Accordingly, only claims 20 and 25 of co-pending Application No. 10/255,568 remain relevant to the instant provisional double patenting rejection. Claims 20 and 25 of co-pending Application No. 10/255,568 are directed to methods of mediating RNA interference using RNAi-mediating compositions (i.e., method claims). The claims of the instant application are, in contrast, directed to RNA interference-mediating compositions (i.e.,

composition of matter claims). Applicants note that in both the instant case and in co-pending Application No. 10/255,568 the Patent Office restricted the method of use claims and composition of matter claims as being directed to patentably distinct inventions. Accordingly, Applicants submit that the instant provisional double patenting rejection in view of claims of co-pending Application No. 10/255,568 is inconsistent with the previous position taken by the Office in both the parent and continuing application.

Co-pending Application No. 10/255,568 is presently undergoing substantive examination. An Office Action mailed June 26, 2007 included a provisional double patenting rejection (obviousness type) of claims directed to methods of mediating RNAi in view of the RNAi-mediating composition of matter claims of the instant invention. Again, Applicants are curious as to the inconsistent position taken by the Office is each of the instant case and the continuing case, *i.e.*, an initial finding of patentable distinctiveness between the composition of matter and methods for the purposes of restriction practice followed by an assertion of obviousness during substantive prosecution. Irrespective of the merits of the Office's position, however, Applicants understand that procedurally, in accordance with Section 804 of the MPEP, a "provisional" double patenting rejection will continue to be made by the Examiner in each case until the "provisional" double patenting rejection is the only rejection remaining in one of the applications. Should the instant claims be found allowable and Applicants are unable to overcome any remaining provisional obvious-type double patenting rejection in co-pending a-Application No. 10/255,568, Applicants will consider filing of a terminal disclaimer in the later-filed case to overcome the rejection.

With regards to Application No. 11/142,866, Applicants note that the pending claims are directed to methods of chemically synthesizing interfering RNAs having recited structural features. By contrast, the claims of the instant case are directed to a broad genus of interfering RNAs that can be produced by a variety of materially distinct processes. Accordingly, Applicants respectfully submit that the provisional double patenting rejection in the instant case is substantively without merit and request withdrawal of the rejection.

Applicants respectfully request that the Examiner hold the provisional double patenting rejections in abeyance. Should the Examiner maintain the provisional double patenting rejections as

the sole remaining rejections in the instant application, Applicant's respectfully request that the rejection be withdrawn as provided by MPEP section 804, subsection I. B., which states:

> "If the "provisional" ODP rejections in two applications are the only rejections remaining in those applications, the examiner should then withdraw the ODP rejection *in the earlier filed application thereby permitting that application to issue* without need of a terminal disclaimer."

***Claim Objections***

Claim 106 is objected to under 37 CFR 1.75(c) as being of improper dependent form for failing to further limit the subject matter of claims 76-78, 81, 86-88, and 91. Specifically, the Examiner objects to the term "complementary to", as allegedly equivalent to the phrase "sequence correspondence to". Applicants respectfully disagree with the Examiner. Nevertheless, in order to more clearly indicate that claim 106 is further limiting, Applicants have amended the claim to replace the phrase "complementary to" with "**perfectly** complementary to". Support for this amendment can be found at least at, for example, at page 2, line 21 through page 3, line 20, in particular, at page 3, lines 9-11 and 17-19, of the specification as originally filed. Accordingly, Applicants submit that amended claim 106 further limits the subject matter of amended claims 76-78, 81, 86-88, and 91.

***Claim Rejections – 35 USC § 112***

Claims 76-78, 81, 86-88, 91, 106, 108, 110, 112 and 115-123 are rejected under 35 U.S.C. 112, second paragraph, for alleged indefiniteness. In particular, the Examiner alleges that the phrase "correspondence to an mRNA" is unclear.

Applicants have amended claims 76-78, 81, 86-88, 91, 106, 108, 110, 112 and 115-123 to more particular point out that isolated RNA molecules of the claimed invention must have "sequence correspondence to an mRNA ***to mediate*** RNA interference by directing cleavage of the mRNA." Support for this amendment can be found at least at, for example, at page 2, line 21 through page 3, line 20, in particular, at page 3, lines 9-11 and 17-19, of the specification as originally filed. Applicants respectfully submit that this phrase is sufficiently clear to one of skill in the art to reasonably apprise them of the scope of the claimed invention, particularly in view of

the teachings of the specification as a whole. Although isolated RNA molecules having sequences with less than perfect sequence correspondence are within the scope of the invention, these RNA molecules must nevertheless have sufficient sequence correspondence to an mRNA to mediate its cleavage by RNAi. Applicants note that the specification sets forth detailed assays for evaluating the ability of a small RNA molecule to mediate RNAi of a target mRNA of interest (see, *e.g.*, Examples 3-5 of the specification). Similar assays are currently and routinely used by those of skill in the art for the same purpose (see, *e.g.*, Ui-Tei *et. al.*, Nucleic Acids Research, 2004, Vol. 32, No. 3 936-948). Accordingly, Applicants respectfully request that this rejection be reconsidered and withdrawn.

Claims 86, 88, 106, 108, 112 and 115-123 are further rejected under 35 U.S.C. 112, second paragraph, for use of the phrase "wherein cleavage is directed within the region of sequence correspondence". In particular, the Examiner alleges that the antecedent basis for "cleavage" in the wherein clause at the end of each claim is unclear. Applicants have amended claims 86, 88, and 112 (and claims 106, 108, 115-123 dependent thereon) to more clearly distinguish cleavage *of the mRNA* from cleavage of double-stranded RNA. Accordingly, reconsideration and withdrawal of this rejection is respectfully requested.

***Claim Rejections – 35 USC § 102/103***

Claims 76-78, 81, 86-88, 91, 106, 108, and 117 are rejected under 35 U.S.C. 102(b) as being anticipated by, or in the alternative, under 35 U.S.C. 103(a) as obvious, over Manche *et al.*, *Molecular and Cell Biology*, (1992), 12: 5238-5248 (herein "Manche"), as evidenced by Stratagene pBluescript II Phagemid Vectors Instruction Manual for Catalog #212207 (herein "Bluescript Manual"), and a Basic Local Alignment Search Tool (BLAST) analysis of the nucleic acid sequence CCCGGTACCCAGCTTTTGTTCCC (herein "BLAST analysis"). In particular, the Examiner alleges that Manche teaches an isolated 23 nucleotide double stranded RNA (herein, the "Manche molecule"). While the Examiner acknowledges that Manche is silent as to any RNA interference properties, the Examiner is of the opinion that the Manche RNA molecule inherently possesses all the essential structural features necessary to mediate RNA interference.

Applicants traverse the rejection.

Applicants first note that the Manche reference is silent with respect to any structural features of the "23-bp duplex RNA" referenced therein. Rather, the reader of the Manche reference is required to dissect the "Materials and Methods" section of the reference describing the making of the duplex RNAs described therein to arrive at the presumed structure of the duplex RNAs. Such an analysis was performed by the Examiner to arrive at the presumed nucleotide sequence of the "23-bp duplex RNA" alleged to *inherently* be an RNAi-mediating molecule according to Applicants' pending claims. Applicants respectfully assert, however, that the Examiner has not looked in sufficient detail at the methodology used by Manche to generate the "23-bp duplex RNA" to fully appreciate the molecule's presumed structure. A complete analysis of said methodology clearly demonstrates that the Manche molecule is not an RNAi-mediating molecule according to the claims of the instant invention.

A detailed review of the experimental methods recited in Manche reveals that the Manche molecule lacks the structural features recited in the claims of the instant application and lacks further structural features necessary for mediating RNAi as required by the instant claims. For the Examiner's convenience, Applicants have prepared a schematic diagram outlining the experimental methods used by Manche to generate the Manche molecule, as well as the structure of the Manche molecule that necessarily resulted therefrom (see Exhibit A).

As the Examiner notes, Manche states that "duplexed RNAs of defined sizes were made by annealing a 358-nt. Transcript synthesized by T7 RNA polymerase with complementary transcripts of various lengths synthesized by T3 RNA polymerase." As stated in the Materials and Methods section of Manche, the DNA template for each *in vitro* transcription reaction consisted of the multiple cloning site region of a pBluescript plasmid vector (pBSII KS+). Specifically, the 358-nt T7 transcript was generated from the plus strand of a plasmid that had been pre-digested by the restriction endonuclease PvuII and the T3 transcripts were generated from the opposite strand of a plasmids that were pre-digested with one of a variety of restriction endonucleases (*i.e.*, Eag I, BamH1, EcoR1, HindIII, Sal I, Xho I, KpnI ,and HaeIII -see Figure 1 of Exhibit A). In the case of

the "23 bp duplex RNA" the T3 transcript was generated with HaeIII. Due to the proximity of the T3 promoter and the HaeIII site in the multiple cloning site, a 23 nucleotide transcript is produced by T3 polymerase.

Manche further states that the T3 and T7 transcripts were annealed and trimmed with RNase A and RNase T1. Annealing the T3 and T7 transcripts would therefore have generated a 23-nt / 358-nt RNA duplex with long single-stranded tails that are subsequently trimmed with RNAse A and RNAse T1 (see Figure 2A of Exhibit A). It is well known that RNAse A specifically attacks single-stranded RNA 3' to pyrimidine residues (*i.e.*, C's and U's) and cleaves the phosphate linkage to the adjacent nucleotide (see page 5.81 of Maniatis et al. which is submitted herewith as Exhibit B), while RNAse T1 specifically attacks the 3'-phosphate groups of guanine nucleotides and cleaves the 5'-phosphate linkage to the adjacent nucleotide (see page 5.82 of Maniatis which is submitted herewith as Exhibit B). Accordingly, due to the known single-stranded specificity of these enzymes, trimming of the 23-nt/358-nt duplex molecule would result in a shortened molecule having the "tails" trimmed in a duplex molecule. It is not clear to what extent the tails would be trimmed. In particular, it is not clear that the single strand-specific RNAses would be able to trim nucleotides within the single stranded portion of the T3/T7 hybrid which are directly adjacent to the double stranded potion of the molecule (*i.e.*, whether the active site of RNAse A or RNAse T1 can accommodate single stranded template having the added complexity in structure due to the double stranded portion). However, even assuming *arguendo* that the single strand-specific RNAses can trim all the way up to the double stranded portion of the annealed T3/T7 transcripts, the shortest molecule produced could only have been a molecule consisting of a 23-nt T3 transcript and a **24-nt** T7 transcript (see Figure 2B of Exhibit A). Thus, although Manche et al. refer to a short "23-bp duplex RNA", the skill artisan would clearly appreciate that the shortest possible molecule produced would be a 23-nt/24-nt duplex. Such a **23-nt / 24-nt duplex** would not share the structural features of the claimed molecules. In contrast to the Manche "23-bp duplex RNA", ***each strand of the claimed dsRNA molecules are 21 to 23 nucleotides in length.*** Accordingly, for at least this reason, the Manche disclosure does not anticipate the claimed invention.

At page 11, paragraph 3 of the Office Action, the Examiner nevertheless appears to be taking the position that the Manche "23-bp duplex RNA" somehow anticipates the claimed siRNA molecules of the invention based on the observation by Manche et al. that "when analysed in denaturing conditions (Fig 1B), the individual strands of the dsRNA molecules were slightly heterogeneous, with chain lengths a few nucleotides longer or shorter than the input single strands as a result of the trimming process" (see p. 5240 of Manche *et al.*). It is critical to note, however, that the "strand heterogeneity" described by Manche et al. is not observed for all of the dsRNA molecules made. While one can see strand heterogeneity for the larger dsRNA molecules of Manche et al. (*i.e.*, the dsRNAs in which the T3 transcript is made by transcribing EagI-, BamHI-, EcoRI-, HindIII-, SalI-, or XhoI-digested template, lanes 1-6 of Fig 1B, respectively), no strand heterogeneity is observed for the dsRNA molecules in which the T3 transcript is made by transcribing HaeIII- or KpnI-digested template. The autoradiograph depicted in Fig 1B of Manche *et al.* clearly shows no strand heterogeneity for the dsRNAs in lanes 7 and 8 (i.e., the HaeIII and KpnI lanes).

The observed strand heterogeneity for the large dsRNAs of Manche et al. is predictable based on the fact that each of these restriction enzymes produces a double-stranded DNA template having a single-stranded, 5' overhanging end (see Figure 3A of Exhibit A). T3 RNA polymerase transcribes such dsDNAs 5'→3'starting at the T3 promoter. There is, however, no terminator sequence. Accordingly, when the T3 polymerase encounters the single-stranded, 5' overhanging end of its dsDNA template, it "runs off" the double-stranded template portion into the single-stranded template portion, creating a heterogeneous population of "run-off" T3 transcripts which vary in length and in the composition of their 3' ends (see Figure 3A of Exhibit A). Additional heterogeneity is caused by the tendency of the phage RNA polymerase to indiscriminately add non-templated nucleotides to the 3' end of the transcript, a phenomenon known as "N+1 addition" (N+1 addition is well documented property of T7 polymerases -see, e.g., Milligan *et al.*, Nucleic Acids Research, (1987), 15: 8783-98). The heterogeneous population of T3 transcripts is then hybridized to long T7 transcript strands. These duplexes are then cleaved with RNAse T1 and RNAse A resulting in dsRNAs having strands a few nucleotides shorter or longer as a result of the trimming process.

Take the dsRNA made with a T3 transcript transcribed 5'→3' from Sal I-digested template as an example. The template generated by SalI digestion has a 5' overhanging end, as follows:

```
5′-TCGACCTCGAG...-3′
      GGAGCTC...-5′
```

Because the single-stranded portion of the DNA template results in "run-off" transcription by the T3 polymerase, transcripts of varying length and 3' end heterogeneity are produced, as follows:

```
T3    5′-GGGAA...CUCGAGG-3′      (36 nt)
T3    5′-GGGAA...CUCGAGGU-3′     (37 nt)
T3    5′-GGGAA...CUCGAGGUC-3′    (38 nt)
T3    5′-GGGAA...CUCGAGGUCG-3′   (39 nt)
T3    5′-GGGAA...CUCGAGGUCGA-3′  (40 nt)
```

Moreover, non-templated nucleotides may be added to the 3' termini of any of the above molecules by the "N+1" activity of T3 polymerase, resulting in molecules of longer or intermediate lengths.

Hybridization of each T3 transcript to the 358 nt transcript produced by T7 polymerase then results in a heterogeneous population of duplexes having a short T3 transcript strand of various lengths and a long T7 transcript strand of uniform length. These duplexes are subsequently trimmed with RNAse T1 and/or A to remove single stranded tails, for example:

```
T3       5′-GGGAA...CUCGAGG-3′        (36 nt)
T7  3′-...UUUCCCUU...GAGCUCCAGCUG...-5′  (358 nt)
         ^^                  ^^^^

T3       5′-GGGAA...CUCGAGGU-3′       (37 nt)
T7  3′-...UUUCCCUU...GAGCUCCAGCUG...-5′  (358 nt)
         ^^                  ^^^^

T3       5′-GGGAA...CUCGAGGUC-3′      (38 nt)
T7  3′-...UUUCCCUU...GAGCUCCAGCUG...-5′  (358 nt)
         ^^                   ^^^
```

```
T3        5′-GGGAA...CUCGAGGUCG-3′    (39 nt)
T7  3′-...UUUCCCUU...GAGCUCCAGCUG...-5′  (358 nt)
          ^^                      ^^

T3        5′-GGGAA...CUCGAGGUCGA-3′    (40 nt)
T7  3′-...UUUCCCUU...GAGCUCCAGCUG...-5′  (358 nt)
          ^^                      ^
```

By contrast, when the T3 RNA polymerase encounters either a blunt end or a single-stranded 3' overhang at the end of the digested dsDNA template, a homogeneous population of T3 transcripts is produced. This is because the 5' terminus of the DNA template strand is entirely double-stranded such that "run-off" transcription (and N+1 addition) is therefore not possible (see Figure 3B of Exhibit A). Accordingly, the T3 transcripts are a homogeneous population of the same length. Hybridization with long T7 strands and subsequent RNAse T1 and/or RNAse A trimming then results in a ***homogeneous population of dsRNAs***.

The dsRNA made with T3 transcript transcribed from HaeIII-digested template is a most relevant example. The template generated by HaeIII digestion has a blunt end, as follows:

```
5′-CCCGG-3′
3′-GGGCC-5′
```

The T3 RNA polymerase terminates at the end of the dsDNA template portion, resulting in transcripts having a uniform length of 23 nucleotides:

```
T3    5′-GGGAA...CCGGG-3′     (23 nt)
```

Hybridization to T7 transcript then results in duplexes having a short 23 nt T3 transcript strand and a long 358 nt T7 transcript strand followed by trimming with the single-stranded RNA specific RNAses T1 and A to remove single stranded tails (see Figure 2A of Exhibit A), for example:

```
T3        5′-GGGAA...CCGGG-3′          (23 nt)
T7  3′-...UUUCCCUU...GGCCCGGGG...-5′   (358 nt)
          ^^                ^^^^
```

As is clear from the above discussion, one observes predictable strand heterogeneity for dsRNAs in which the input T3 strands are heterogeneous and ***predictable homogeneity*** for dsRNAs in which the input T3 strands are homogeneous (*e.g.*, Hae III-digested template strands).

Furthermore, 23-nt. / 23-nt. duplex molecule, none of the duplex RNA molecules made according to the procedure of Manche et al. would have the appropriate chemical composition to mediate RNA interference. Particular attention is directed to the chemical composition of the 5'end of each strand of the Manche molecule (Figure 2B of Exhibit A). Applicants submit that the T7 transcript strand would necessarily have had a **5' triphosphate moiety** (*i.e.*, 5'-P-P-P-), since it is known in the art that T7 phage RNA polymerase generates a transcript having a triphosphate moiety at its 5' end (see Page 5.59 from Maniatis et al., which is submitted herewith as Exhibit B). Moreover, Applicants submit that the T3 transcript strand would necessarily have had a **5'-hydroxyl moiety** (*i.e.*, 5-OH-), since RNase T1 is known to generate cleavage products having 3'-phosphate and 5'-hydroxyl residues at their cleaved termini.

Due to the chemical composition of their 5' ends, neither the T7 transcript strand nor T3 transcript strand of the Manche molecule would be expected to mediate RNA interference. It is now well-known that a single 5' phosphate (*ie.*, a 5'-***mono***phosphate) is required for small RNA to function in mediating RNA interference (*see*, *e.g.*, Nykänen *et al.*, *Cell,* 107: 309-321 (2001) submitted herewith as Exhibit C). For example, the cell employs ATP to maintain 5' monophosphates on small RNAs and ensure recognition by RNAi enzymes such as RISC (see Nykänen, *id.*). Recent investigations into the structure of the Piwi-domain of RISC has revealed that its selectivity for the small RNAs of the invention is in part due to a binding pocket that is finely tuned to bind the 5' monophosphate of these small RNAs, while excluding other RNAs that lack this feature (see, *e.g.*, Parker *et al., Nature,* 434: 663 (2005) and Ma *e al., Nature,* 434: 666 (2005) submitted herewith as Exhibits D and E, respectively). Indeed, the specification recognizes

the importance of ATP, and therefore phosphorylation, in mediating RNA interference (see Example 2 entitled "Double-Stranded RNA Directs the ATP-Dependent Cleavage of mRNA at 21 to 23 Nucleotide Intervals").

Finally, the Manche molecule suffers from additional sequence characteristics which would likely render it inoperable as an siRNA even if it was engineered to have the appropriate termini and length characteristics of the molecules of the invention. In particular, the Examiner's attention is drawn to the G/C content at the ends of the Manche molecule (see Figure 2B of Exhibit A). The first five nucleotides (P1-P5) at both ends of the molecule contain a tract of G/C base pairs which impair the ability of the double-stranded molecule to unwind and load into RISC to subsequently mediate RNAi. It is now well known that the base pair strength at each end of siRNA must be below a threshold of intramolecular free energy (about -10.5 kcal/mol) in order for the strands to unwind and incorporate into RISC (see, *e.g.*, Zamore *et al.*, Cell, (2003), 115: 199-208). However, the stability at both ends of the Manche molecule is above this threshold value. For example, a rough calculation of the free energy at the 5' end of the molecule referencing the T3 strand (5'-GGGAA'-3) reveals a free energy of -11 kcal/mol threshold, while the stability of the 5' end of the molecule referencing the T7 strand (5'-CCCGG-3') is even higher (-12.1 kcal/mol). As such, the high thermal stability of the Manche molecule indicates that it would likely be unable to unwind and mediate RNAi.

Accordingly, in view of the above comments, Applicants submit that the Manche molecule lacks structural features common to the small RNA molecules of the claimed invention. Thus, the pending claims are neither anticipated, nor rendered obvious, by Manche.

## CONCLUSION

In view of the above amendment, applicant believes the pending application is in condition for allowance.

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Respectfully submitted,

By _____

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